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Effects of 5-HT_{1A} Receptor Ligands on a Safety Signal Withdrawal Procedure of Conflict in the Rat

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CHARRIER, D., L. DANGOUMAU, M. HAMON, A. J. PUECH AND M. H. THIÉBOT. *Effects of 5-HT_{1A} receptor ligands on a safety signal withdrawal procedure of conflict in the rat.* PHARMACOL BIOCHEM BEHAV 48(1) 281-289, 1994. – The present study evaluated in the rat the ability of various 5-HT_{1A} receptor agonists to exert an "anxiolytic-like" release of the suppression of lever pressing for food induced by the withdrawal of a conditioned signal for safety without presentation of a conditioned signal for punishment. During the period associated with the safety signal withdrawal (Saf.CS – /Pun.CS –), control rats exhibited a typical pattern of responding with an initial strong blockade of responding that lessened over the period as presses were rewarded and shocks omitted. The 5-HT_{1A} receptor partial agonists buspirone (0.125–0.5 mg/kg) and 8-(2-[2,3-dihydro-1,4-benzodioxin-2-yl-methylamino]ethyl)-8-azaspiro[4,5]decane-7,9-dione methyl sulfonate (MDL 73005EF; 0.5–2 mg/kg) and the full agonist (+)-4-[*N*-(5-methoxy-chroman-3-yl)-*N*-propylamino]-butyl-8-azaspiro[4,5]decane-7,9-dione (S 20499; 0.125–1 mg/kg) produced a robust and dose-related release of pressing during the Saf.CS – /Pun.CS – period. This effect was less marked with ipsapirone (0.125–1 mg/kg). Conversely, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT; 0.06–0.25 mg/kg), a full agonist, was completely inactive and did not prevent MDL 73005EF (1–2 mg/kg) or diazepam (0.125 mg/kg) from releasing the suppressed behavior. The specific 5-HT_{1A} antagonist (+)-*N-tert*-butyl-3-4-(2-methoxyphenyl)piperazin-1-yl-2-phenylpropanamide [(+)-WAY 100135; 0.25–8 mg/kg] and the β-adrenoceptor/5-HT_{1A} antagonist (–)-tertatolol (2–8 mg/kg) did not modify the behavioral blockade, nor did (+)-WAY 100135 (2–4 mg/kg) reduce the ability of buspirone (0.25 mg/kg) to enhance responding during the Saf.CS – /Pun.CS – period. Finally, neither 1-(2-pyrimidinyl)piperazine (1-PP), the common metabolite of azapirones, with α_2-adrenoceptor antagonist properties, nor the D_2-receptor antagonist *l*-sulpiride reduced the behavioral suppression. These data suggest that the activation of somatodendritic and/or postsynaptic 5-HT_{1A} receptors does not entirely account for the anxiolytic-like effects of 5-HT_{1A} agonists in this procedure of conflict in the rat.

5-HT_{1A} receptor agonists Partial agonists and antagonists Anxiety Behavioral suppression Rat

IT was suggested more than two decades ago that serotonergic processes are probably involved in anxiety and in the action of anxiolytic drugs. This hypothesis was based on a variety of data showing that the inhibition of serotonergic transmission releases behavior from blockade induced by unpleasant events, as do benzodiazepines (44,49). Accordingly, benzodiazepines were shown to reduce (amongst other effects) the activity of serotonin (5-HT) neurons (40,48), although other data did not support the view that the anxiolytic properties of benzodiazepines are closely linked to a decrease in serotonergic function (36,39,45).

Recently, the serotonin hypothesis of anxiety gained renewed interest with the discovery that some drugs which have clinical anxiolytic activity interact directly with 5-HT receptors. In particular, this is the case for buspirone and related azapirones such as gepirone and ipsapirone, which act as 5-HT_{1A} receptor agonists (21). However, it should be noted that none of these compounds have been unequivocally demonstrated to be as efficient as benzodiazepines in rodent and monkey procedures used to predict an anxiolytic activity. In fact, when anxiolytic-like effects could be observed, they were frequently smaller and less consistent than those of benzodiaz-

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epines. Moreover, in some studies, 5-HT_{1A} agonists exhibited anxiogenic-like effects (3,14,20,30,46).

We have developed a new experimental paradigm in the rat in which a blockade of lever pressing for food was induced by the withdrawal of a conditioned signal for safety without presentation of a conditioned signal for danger. As distinct from standard paradigms, in which the behavioral suppression is a learned response to contingent punishment or to a punishment signal, this procedure involves a relative degree of unpredictability to which the animals have no readily prepared appropriate response and which could reflect a different type of anxiety-producing event. In this paradigm, not only benzodiazepines, but also two 5-HT_{1A} agonists, buspirone and gepirone, released the behavioral blockade induced by the withdrawal of the safety signal (43). However, another 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), failed to produce a comparable effect, as also noted in several procedures used to assess anxiety in animals (3,14,46).

Since none of the 5-HT_{1A} agonists are selective ligands for the 5-HT_{1A} sites, these differences could be linked to their respective affinity for other 5-HT receptor subtypes (5-HT_{1B}, 5-HT_{1C}, 5-HT_2); for other neurotransmitter receptors such as the dopaminergic D_2 receptors, the α_1- or α_2-adrenoceptors, or the muscarinic M_1 receptors [see (27,51)]; and/or for sites associated with 5-HT reuptake processes (18). The extensive metabolic transformation of azapirones (buspirone, ipsapirone, and gepirone), but not of 8-OH-DPAT, to 1-(2-pyrimidinyl)piperazine (1-PP), which exhibits potent α_2-adrenoceptor blocking properties (1,16), could also account for the differences in the ability of 5-HT_{1A} agonists to induce anxiolytic-like effects.

In order to further characterize the mechanism(s) of the anxiolytic-like effects of 5-HT_{1A} receptor ligands, several compounds with different intrinsic efficacies at pre- (i.e., somatodendritic) and/or postsynaptic 5-HT_{1A} receptors (41,42) were studied in the safety signal withdrawal procedure. They exhibited either a full agonist activity at both pre- and postsynaptic 5-HT_{1A} receptors (8-OH-DPAT, S 20499) (19,23,38), a partial agonist/antagonist efficacy at the postsynaptic level (ipsapirone, buspirone, MDL 73005EF) (8,15,25,50), or an antagonist activity at pre- and postsynaptic sites, as is the case for the selective antagonist (+)-*N-tert*-butyl-3-4-(2-methoxyphenyl)piperazin-1-yl-2-phenylpropanamide [(+)-WAY 100135] (13) and the β-adrenoceptor/5-HT_{1A} antagonist (−)-tertatolol (22,32). In an attempt to further delineate the respective roles of pre- and postsynaptic 5-HT_{1A} receptors in the ability of drugs to release suppressed behavior, interactions between the full agonist 8-OH-DPAT, or the antagonist (+)-WAY 100135, and a partial agonist (MDL 73005EF or buspirone) have been studied. A putative involvement of 5-HT_{1A}-related processes in the anticonflict activity of benzodiazepines was also assessed by studying the effects of the coadministration of 8-OH-DPAT and diazepam.

METHODS

Animals

The experiments were carried out on male Wistar AF rats (C.E.R.J., Le Genest, France) weighing 100 ± 10 g at the beginning of the training and 300–400 g at the time of the test sessions. They were housed eight per cage under standard laboratory conditions (12-h light–dark cycle, room temperature 21 ± 1°C) with free access to water in their home cage. One week prior to the beginning of the experiments rats were placed on a daily schedule of food restriction (13 g of standard chow per day per rat) which was maintained until the end of the experiments.

Apparatus

The experiments were conducted in four standard ventilated, sound-attenuated operant chambers (Campden Instruments Ltd., UK). Each chamber was fitted with an electrified grid floor and an automatic magazine delivering food pellets (45 mg, Campden) in a tray located between two response levers. The chambers were supplied with three lights (24 V, 3 W) located above each lever and in the middle of the ceiling (house light).

Procedure

The experimental procedure has previously been described in detail (43). It consisted of two successive phases: training sessions and test sessions.

Training sessions. During daily 18-min sessions rats were trained to press the right lever to obtain food pellets according to a fixed-ratio 1 (FR 1) schedule of reinforcement, which was progressively raised to an FR 8 schedule. Pressing the left lever had no scheduled consequences throughout the experimental procedure. The stimulus light situated above the right lever was illuminated during these initial training sessions. After stabilization of FR 8 responding, two 4-min punished periods, signaled by the illumination of the light situated above the left lever (punishment signal = Pun.CS+), were introduced in the course of the sessions. They started 4 min and 11 min after the beginning of the session. During these periods, presses were reinforced with food pellets according to an FR 1 schedule and were also associated with scrambled electric footshocks according to a random ratio 50% (RR 50%) schedule (50 ± 15% of the presses were randomly punished). The shock intensity, initially set at 0.5 mA for 45 ms, was increased gradually and adjusted for each rat to cause a similar degree of response suppression (range 0.5–2 mA). Shock intensity was not modified after punished responding stabilized to a level whereby rats received six shocks or less during the punished periods (total 8 min). The nonpunished periods were signaled by the illumination of the right stimulus light (safety signal = Saf.CS+) as during the initial training. Visual stimuli were maintained throughout the appropriate periods.

The number of pellets obtained and shocks received by each rat were automatically recorded every minute. Approximately 20 sessions after the initiation of the punishment contingency were necessary to obtain stable response baselines—that si, 50–80 presses/min during nonpunished periods (corresponding to 6–10 pellets earned/min) and 0–2 presses/min during punished periods. Drug studies were then initiated.

Test sessions. The 11-min test sessions were organized into three successive periods. Periods 1 (4 min) and 3 (3 min) were signaled by the right stimulus light (Saf.CS+) and associated to a nonpunished FR 8 schedule of food presentation identical to that in effect during the corresponding periods of the training sessions. During period 2 (4 min), the safety signal (right light) was turned off, and the *punishment signal (left light) was not presented* (safety signal withdrawal = Saf.CS−/Pun.CS−). During this period only, the house light was illuminated, each lever press was reinforced by a food pellet (FR 1), and *no shocks were delivered.*

Rats were subjected to no more than seven test sessions, and drug treatments were administered at intervals of at least

seven days. Between test sessions, animals were subjected to at least four additional training sessions. For each test session, the rats were divided into groups of 7–12 animals, matched according to their performances during the last training session. Group matching was done on the basis of the total number of pellets earned, the total number of shocks received, and the percentage of presses that were associated with shocks during the punished periods. Rats were subjected to daily IP or SC saline injections for at least four days before receiving the test drugs. For several compounds, the complete range of doses was studied in the course of two independent test sessions. In this case, a group of saline-injected animals was associated with each test session. The performances of these independent groups of control rats (which never statistically differed from each other) were pooled for the analysis and the presentation of the results.

Drugs

The drugs used were buspirone-HCl (Bristol Myers, France), ipsapirone-HCl (Troponwerke, Germany), 8-(2-[2,3-dihydro-1,4-benzodioxin-2-yl-methylamino]ethyl)-8-azaspiro-[4,5]decane-7,9-dione methyl sulfonate (MDL 73005EF; Merrell Dow, France), 8-OH-DPAT-HBr (Research Biochemicals Inc., Natick, MA), (+)-4-[*N*-(5-methoxy-chroman-3-yl)-*N*-propylamino]-butyl-8-azaspiro[4,5]decane-7,9-dione (S 20499-HCl; Servier, France), (+)-WAY 100135-2HCl (Wyeth Research, UK), (−)-tertatolol-HCl (Servier), 1-PP-2HCl (Aldrich, France), *l*-sulpiride (Delagrange, France), and diazepam (Hoffmann-La-Roche, Switzerland). Except for diazepam and *l*-sulpiride, which were suspended in acacia gum, the drugs were dissolved in saline (0.9% NaCl). As appropriate, the doses are expressed as the salt or the base. Drugs or vehicle were administered IP or SC in a volume of 0.5 ml/100 g body weight.

Statistical Analyses

Drug effects were analyzed independently on the number of pellets obtained during the nonpunished (Saf.CS+) periods 1 and 3 (total 7 min) and during period 2, associated with the safety signal withdrawal (Saf.CS−/Pun.CS−), by one-way analysis of variance (ANOVA) or two-way ANOVA for drug interactions. Planed pairwise comparisons between groups were made using two-tailed Dunnett's or Dunn's *t* test using the appropriate error variance term from ANOVAs.

RESULTS

As previously described (43), during periods 1 and 3 of the test sessions saline-injected rats exhibited a high level of lever pressing, corresponding to an average of 6–10 pellets obtained per minute. These performances did not differ from that observed during the nonpunished periods of the training sessions. During period 2, associated with the safety signal withdrawal without presentation of the punishment signal (Saf.CS−/Pun.CS−), control animals typically exhibited a blockade of responding that lessened over the 4-min period as shocks were omitted (see control group in Fig. 1).

Buspirone (0.125–0.5 mg/kg SC), as previously observed after IP administration (43), significantly enhanced the number of pellets obtained during period 2 associated with the safety signal withdrawal, $F(3, 24) = 5.94$, $p < 0.005$. This was due to a significant effect induced by the three doses studied, 0.125 mg/kg (+209%, $p < 0.05$), 0.25 mg/kg (+320%, $p < 0.01$), and 0.5 mg/kg (+215%, $p < 0.05$).

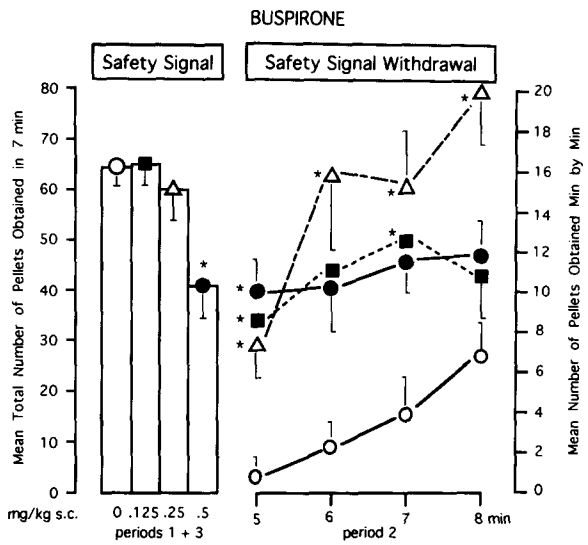

FIG. 1. Effects of buspirone on the number of pellets obtained during the two components of the safety signal withdrawal operant schedule. Histograms represent the total number of pellets earned under a fixed-ratio 8 (FR 8) schedule of food reinforcement, during periods 1 and 3 (total 7 min) associated with the safety signal. Curves represent the number of pellets obtained minute by minute under an FR 1 schedule of food delivery, during period 2 (4 min) associated with the safety signal withdrawal (note the different scales). Buspirone or vehicle was injected SC 30 min before the test. ○ controls, ■ 0.125 mg/kg, △ 0.25 mg/kg, ● 0.5 mg/kg ($n = 7$ per group) (for clarity of the figure, some SEM bars have been omitted). *$p < 0.05$ as compared to controls during the same period (Dunnett's *t* test after ANOVA).

During periods 1 and 3, the total number of pellets earned was significantly reduced by buspirone 0.5 mg/kg (−37%, $p < 0.05$) (Fig. 1).

Ipsapirone (0.125–1 mg/kg SC) had a significant overall effect on performance during period 2, $F(4, 49) = 2.59$, $p < 0.05$. Pairwise comparisons revealed that this was due to a significant increase of the number of pellets obtained only at the dose of 0.5 mg/kg (+145%, $p < 0.05$). Responding during periods 1 and 3 was significantly reduced ($p < 0.05$) by ipsapirone 1 mg/kg (Table 1).

MDL 73005EF (0.5–2 mg/kg SC) had a significant overall effect on the number of pellets obtained during period 2, $F(3, 24) = 6.08$, $p < 0.005$. This was due to a significant increase induced by 1 mg/kg (+135%, $p < 0.05$) and 2 mg/kg (+175%, $p < 0.01$). MDL 73005EF (2 mg/kg) significantly reduced (−41%, $p < 0.05$) performance during periods 1 and 3 (Table 1).

8-OH-DPAT (0.06–0.25 mg/kg IP) did not significantly modify lever pressing during period 2 associated with the safety signal withdrawal, $F(3, 56) = 1.36$, NS, as already observed after SC injections (43). Responding during periods 1 and 3 was not significantly changed by these doses of 8-OH-DPAT (Table 1).

When coadministered with effective doses of MDL 73005EF (1 and 2 mg/kg SC) or diazepam (1 mg/kg IP), 8-OH-DPAT (0.125 mg/kg SC) did not significantly alter the increase in lever pressing induced by either drug during the safety signal withdrawal component: MDL, $F(2, 36)_{int} = 0.89$, NS; diazepam, $F(1, 52)_{int} = 0.54$, NS (Table 2). During

TABLE 1

EFFECTS OF 5-HT$_{1A}$ RECEPTOR LIGANDS ON THE NUMBER OF PELLETS OBTAINED DURING THE TWO COMPONENTS OF THE OPERANT SCHEDULE

			Total Number (mean ± SEM) of Pellets Obtained During Periods Associated With	
	mg/kg	*n*	Safety Signal	Safety Signal Withdrawal
Saline	–	16	61 ± 3	12.7 ± 2.5
Ipsapirone	0.125	7	66 ± 6	12.3 ± 4.7
SC 15min	0.25	17	61 ± 3	23.1 ± 3.7
	0.5	7	58 ± 3	31.1 ± 9.0*
	1	7	49 ± 2*	26.7 ± 8.2
Saline	–	7	68 ± 5	16.7 ± 3.4
MDL 73005EF	0.5	7	66 ± 6	27.9 ± 5.1
SC 30min	1	7	53 ± 3	39.3 ± 7.4*
	2	7	40 ± 4†	45.6 ± 3.7†
Saline	–	20	57 ± 4	13.7 ± 2.0
8-OH-DPAT	0.06	10	56 ± 3	12.6 ± 2.9
IP 60min	0.125	20	52 ± 2	18.7 ± 2.4
	0.25	10	53 ± 3	14.6 ± 2.0
Saline	–	18	65 ± 2	8.1 ± 2.4
S 20499	0.125	8	62 ± 3	14.9 ± 3.6
SC 30min	0.25	8	47 ± 5†	19.0 ± 3.9
	0.5	8	46 ± 2†	25.4 ± 4.9*
	1	18	38 ± 2†	30.7 ± 4.3†
Saline	–	18	66 ± 3	14.8 ± 3.6
(+)-WAY 100135	0.25	8	62 ± 3	10.1 ± 3.2
SC 30min	0.5	8	62 ± 5	11.6 ± 4.3
	1	8	67 ± 5	7.4 ± 2.2
	2	8	65 ± 3	17.4 ± 7.8
	4	8	61 ± 5	8.3 ± 1.1
	8	8	50 ± 4*	15.9 ± 3.9
Saline	–	7	66 ± 5	7.9 ± 2.9
(−)-Tertatolol	2	7	66 ± 3	4.6 ± 0.9
IP 30min	4	7	65 ± 5	7.1 ± 2.4
	8	7	66 ± 3	9.2 ± 3.6

Results are the total number of pellets earned under a fixed-ratio 8 (FR 8) schedule of food reinforcement, during periods 1 and 3 (total 7 min) associated with the safety signal, and under an FR 1 schedule of food delivery, during period 2 (4 min) associated with the safety signal withdrawal. *$p < 0.05$, †$p < 0.01$ vs. associated saline group during the same period (Dunnett's *t* test after ANOVA).

periods 1 and 3, the coadministration of 8-OH-DPAT (0.125 mg/kg) with MDL (1 mg/kg) or diazepam (1 mg/kg) induced a significant ($p < 0.05$) reduction in the number of pellets obtained by rats as compared to the associated drug alone. At this dose, 8-OH-DPAT occasionally lessened performances during these two periods associated with the safety signal (Table 2).

S 20499 (0.125–1 mg/kg SC) had a significant overall effect on the number of pellets obtained during period 2, $F(4,55) = 6.79$, $p < 0.001$. Pairwise comparisons showed that this was due to a significant increase induced by 0.5 mg/kg (+214%, $p < 0.05$) and 1 mg/kg (+279%, $p < 0.01$). S 20499 (0.25, 0.5, and 1 mg/kg) caused a significant reduction in responding (−28%, −29%, and −42%, respectively; $p < 0.05$) during periods 1 and 3 (Table 1).

(+)-WAY 100135 (0.25–8 mg/kg SC) did not significantly change the number of pellets obtained during period 2, $F(6, 59) = 0.75$, NS. The highest dose studied (8 mg/kg) significantly reduced (−24%, $p < 0.05$) performance during periods 1 and 3 (Table 1). (+)-WAY 100135 (4 mg/kg SC) significantly antagonized the increase in lever pressing induced by buspirone (0.125 mg/kg SC) but not the increase induced by buspirone (0.25 mg/kg SC) during the safety signal withdrawal component, $F(1, 28)_{int} = 5.83$, $p < 0.05$ and $= 0.01$, NS, respectively. Performance during periods 1 and 3 was not modified by the coadministration of (+)-WAY 100135 and buspirone (Table 3).

(−)-Tertatolol (2–8 mg/kg IP) did not significantly modify responding during period 2, $F(3, 24) = 1.18$, NS, and periods 1 and 3 (Table 1).

1-PP (0.5–4 mg/kg IP) did not induce significant variations in the number of pellets obtained during period 2, $F(4, 50) = 1.48$, NS, and periods 1 and 3 (Table 4).

l-*Sulpiride* (0.25–4 mg/kg IP) did not significantly modify

TABLE 2

INTERACTION OF 8-OH-DPAT WITH MDL 73005EF OR DIAZEPAM ON THE NUMBER OF PELLETS OBTAINED DURING THE TWO COMPONENTS OF THE OPERANT SCHEDULE

Compounds (mg/kg)			Total Number (mean ± SEM) of Pellets Obtained During Periods Associated With	
(60 min)	(30 min)	*n*	Safety Signal	Safety Signal Withdrawal
Saline	Saline	7	58 ± 7	14.7 ± 5.8
8-OH-DPAT (0.125)	Saline	7	53 ± 3	12.3 ± 2.9
Saline	MDL (1)	7	56 ± 2	44.0 ± 5.9†
8-OH-DPAT (0.125)	MDL-(1)	7	38 ± 5*‡	36.1 ± 4.4†
Saline	MDL (2)	7	38 ± 5*	25.1 ± 2.6*
8-OH-DPAT (0.125)	MDL (2)	7	33 ± 4*	28.7 ± 2.7*
Saline	Saline	14	66 ± 2	13.3 ± 2.9
8-OH-DPAT (0.125)	Saline	14	43 ± 2†	17.7 ± 3.7
Saline	DZP (1)	14	64 ± 3	37.8 ± 4.3†
8-OH-DPAT (0.125)	DZP (1)	14	42 ± 3*§	38.9 ± 5.1†

Results are the total number of pellets earned under a fixed-ratio 8 (FR 8) schedule of food reinforcement, during periods 1 and 3 (total 7 min) associated with the safety signal, and under an FR 1 schedule of food delivery, during period 2 (4 min) associated with the safety signal withdrawal. 8-OH-DPAT and MDL 73005EF (MDL) were injected SC and diazepam (DZP) was given IP. *$p < 0.05$, †$p < 0.01$ vs. associated saline–saline group during the same period. ‡$p < 0.05$, §$p < 0.01$ vs. associated group given MDL or diazepam alone (Dunn's *t* test after ANOVA).

the number of pellets obtained during period 2, $F(5, 78) = 0.15$, NS, and periods 1 and 3 (Table 4).

DISCUSSION

As compared to standard conflict procedures, the present one is original in its method of generating a behavioral suppression, since it is the elimination of the contingency stimuli—the withdrawal of the signal for safety—that is likely to play a crucial role. This event has no conditioned significance and rats have no known appropriate response to it. This represents a complex, though moderate, stressful stimulus which probably enhances the state of arousal and, among other neurobiological functions, the activity of 5-HT neurons. It has

TABLE 3

INTERACTION OF (+)-WAY 100135 WITH BUSPIRONE (BUS) ON THE NUMBER OF PELLETS OBTAINED DURING THE TWO COMPONENTS OF THE OPERANT SCHEDULE

Compounds (mg/kg, SC)			Total Number (mean ± SEM) of Pellets Obtained During Periods Associated With	
(35 min)	(30 min)	*n*	Safety Signal	Safety Signal Withdrawal
Saline	Saline	8	61 ± 3	9.3 ± 3.8
WAY (4)	Saline	8	53 ± 2	11.5 ± 5.9^{ns}
Saline	Bus (0.125)	8	59 ± 5	38.1 ± 7.6†
WAY (4)	Bus (0.125)	8	50 ± 3	13.6 ± 4.1*^{ns}
Saline	Saline	8	71 ± 4	13.8 ± 6.0
WAY (4)	Saline	8	63 ± 2	6.0 ± 1.8^{ns}
Saline	Bus (0.25)	8	70 ± 5	43.3 ± 9.2†
WAY (4)	Bus (0.25)	8	63 ± 4	36.9 ± 5.2‡^{NS}

Results are the total number of pellets earned under a fixed-ratio 8 (FR 8) schedule of food reinforcement, during periods 1 and 3 (total 7 min) associated with the safety signal, and under an FR 1 schedule of food delivery, during period 2 (4 min) associated with the safety signal withdrawal. ns = nonsignificant vs. saline–saline group during the same period. †$p < 0.01$ vs. saline–saline group during the same period. *$p < 0.05$ vs. associated group given Bus alone (Dunn's *t* test after ANOVA). ‡$p < 0.05$ vs. saline–saline group during the same period. NS = nonsignificant vs. associated group given Bus alone (Dunn's *t* test after ANOVA).

TABLE 4

EFFECTS OF 1-(2-PYRIMIDINYL)PIPERAZINE (1-PP) AND *l*-SULPIRIDE ON THE NUMBER OF PELLETS OBTAINED DURING THE TWO COMPONENTS OF THE OPERANT SCHEDULE

			Total Number (mean ± SEM) of Pellets Obtained During Periods Associated With	
	mg/kg	*n*	Safety Signal	Safety Signal Withdrawal
Saline	–	13	66 ± 4	17.7 ± 2.7
1-PP	0.5	8	61 ± 4	21.0 ± 3.3
IP 30 min	1	13	68 ± 3	25.8 ± 3.8
	2	13	63 ± 3	23.8 ± 5.8
	4	8	63 ± 3	17.6 ± 5.0
Saline	–	18	64 ± 3	14.9 ± 3.2
l-Sulpiride	0.25	18	62 ± 2	15.7 ± 3.3
IP 30 min	0.5	10	65 ± 2	13.6 ± 3.5
	1	18	63 ± 3	16.5 ± 3.2
	2	10	64 ± 3	15.1 ± 4.1
	4	10	64 ± 3	18.3 ± 4.8

Results are the total number of pellets earned under a fixed-ratio 8 (FR 8) schedule of food reinforcement, during periods 1 and 3 (total 7 min) associated with the safety signal, and under an FR 1 schedule of food delivery, during period 2 (4 min) associated with the safety signal withdrawal. No significant difference vs. associated saline group during the same period (ANOVA).

been proposed, for instance, that the reduction in 5-HT transmission due to 5-HT_{1A} agonists acting at somatodendritic receptors could be more effective against anxiety associated with unpredictable or uncontrollable stress than against anxiety associated with predictable aversive events (2,4).

The behavioral suppression induced by the withdrawal of the signal for safety (Saf.CS – /Pun.CS –) was markedly released by buspirone, as previously obtained using a different route of administration (43). MDL 73005EF, a partial agonist/antagonist at 5-HT_{1A} postsynaptic receptors (15,50), also clearly enhanced responding during the Saf.CS – /Pun.CS – period. The effect of the two substances reached the same magnitude as that obtained with benzodiazepines (43). Ipsapirone, which was described as a full or partial agonist (19,25), produced a similar but less marked effect, as was the case with gepirone in the first study (43). Finally, 8-OH-DPAT did not release the behavioral blockade, whatever the route of administration [present results, (43)]. It must be pointed out, however, that although the procedure was able to detect either a release or a further blockade of behavior under identical experimental conditions, 8-OH-DPAT never induced an "anxiogenic-like" effect as reported in other situations such as the elevated plus-maze (6). Therefore, the 5-HT_{1A} receptor ligands are not equipotent in their ability to exert an anxiolytic-like activity in the safety signal withdrawal conflict paradigm.

Before further considering 5-HT-related mechanisms in these effects, one must examine whether the observed differences might be linked to other processes. Indeed, 1-PP, the common metabolite of azapirones, is a potent α_2-adrenoceptor antagonist, which may account for the anxiolytic-like effects of these drugs [e.g., see (17)]. However, whereas yohimbine was found to release suppressed behavior in the safety signal withdrawal paradigm (M.H.T., unpublished results), 1-PP itself, over a large range of doses, failed to enhance responding during the Saf.CS – /Pun.CS – period. Furthermore, active compounds such as S 20499 and MDL 73005EF are not catabolized to 1-PP. Therefore, the hypothesis of an involvement of α_2-adrenoceptor blockade in the anxiolytic-like effect of 5-HT_{1A} agonists seems unlikely.

Despite their high affinity for 5-HT_{1A} receptors, it cannot be excluded that the active compounds produced their behavioral effects through sites such as the dopaminergic D_2 receptors, for which they exhibit some affinity. Indeed, not only buspirone, but also ipsapirone, gepirone, MDL 73005EF, and S 20499 bind to D_2 receptors with a micromolar affinity and/or modify central dopamine turnover (19,23,51). Both effects could be sufficient to account for the observed variations in behavioral blockade [e.g., see (26)]. Indeed, the doses active in releasing the suppressed behavior are generally higher than those which reduce the firing of 5-HT neurons in vivo (5,23). However, since the specific D_2 receptor antagonist l-sulpiride was not active in the present study, it seems unlikely that blockade of D_2 receptors would constitute the main mechanism of the anxiolytic-like effects of some 5-HT_{1A} receptor ligands in the safety signal withdrawal procedure.

Presynaptic events (i.e., stimulation of the somatodendritic autoreceptors resulting in a reduction in 5-HT transmission) have been proposed to play a crucial role in the anxiolytic-like effects of 5-HT_{1A} agonists. Indeed, 5-HT depletion or selective neurotoxin lesions of dorsal raphe neurons prevented the effects of buspirone-like drugs in several animal tests of anxiety (2,6,9,10). However, in other studies, 5-HT depletion failed to counteract the anxiolytic-like effects of 5-HT_{1A} partial agonists, suggesting a major role for postsynaptic events (7,11, 33,37).

In the present study, the intensity of the behavioral release induced by the various 5-HT_{1A} agonists tested was, at least apparently, inversely related to their intrinsic efficacy at postsynaptic 5-HT_{1A} receptors. Thus, it can be proposed that both pre- and postsynaptic mechanisms are involved. A release of the behavioral suppression induced by the withdrawal of the safety signal would be achieved when a global reduction in

5-HT transmission (due to the stimulation of the somatodendritic receptors) is not counterbalanced by a direct activation of postsynaptic 5-HT_{1A} sites, as seems to be the case with 8-OH-DPAT and, to a lesser extent, with ipsapirone. According to this hypothesis, partial agonists such as MDL 73005EF and buspirone would act as relative antagonists at the postsynaptic level.

Several results, however, do not support the hypothesis that pre- and postsynaptic 5-HT_{1A} receptors may have opposing functions in anxiety-related behavior. The compound S 20499, which was reported to be a full agonist at both pre- and postsynaptic 5-HT_{1A} sites in vitro and in vivo in anesthetized rats (23), did release the behavioral blockade induced by the safety signal withdrawal. This effect occurred at doses which induced motor disturbances as indicated by the decrease in baseline lever pressing. However, it has not been established whether or not S 20499 was effective in inducing behavioral symptoms of postsynaptic 5-HT_{1A} receptor stimulation such as forepaw treading (31,35). However, when they have been observed, these symptoms occurred at doses much larger than those active in the present procedure, which were themselves higher than those necessary to block the firing of 5-HT neurons in the dorsal raphe (23). Therefore, it cannot be excluded that S 20499 or a yet unknown metabolite could exert, at least at low doses, a partial agonist activity at postsynaptic 5-HT_{1A} sites.

According to the proposal that the ability of 5-HT_{1A} ligands to release suppressed behaviour could result from a balance between opposing effects at pre- and postsynaptic receptors, the full agonist 8-OH-DPAT should counteract the effect of a partial agonist/antagonist such as MDL 73005EF. Accordingly, in the punished drinking test 8-OH-DPAT dose-dependently antagonized the MDL 73005EF-induced increase in shocks received (29). In the present paradigm, 8-OH-DPAT injected subcutaneously at a relatively high dose—since it reduced baseline responding in some experiments [see Table 2 and (43)]—and which was able to stimulate postsynaptic 5-HT_{1A} receptors (47) failed to counteract the increase in lever pressing induced by MDL 73005EF during the period associated with the safety signal withdrawal. It must be added that 8-OH-DPAT was also unable to counteract the effects of diazepam in the same procedure. Thus, in the present experimental conditions, a stimulation of the postsynaptic 5-HT_{1A} receptors cannot be viewed as the relevant mechanism to counterbalance an anxiolytic-like effect which would result from a reduction in 5-HT transmission mediated by the activation of either 5-HT_{1A} autoreceptors or $GABA_A$/benzodiazepine/Cl^- receptors.

A third point deserves attention. (+)-WAY 100135 and (−)-tertatolol, which behave as competitive antagonists (specific and nonspecific, respectively) at both pre- and postsynaptic 5-HT_{1A} receptors (13,22,24,32), failed to modify rats' behavior during the period associated with the safety signal withdrawal. This adds a negative result to the very few behavioral studies published to date, indicating that 5-HT_{1A} receptor antagonists exhibited inconsistent (if any) anxiolytic-like effects (12,28). This suggests that in awake, behaving animals, 5-HT does not exert—via 5-HT_{1A} receptors—a tonic influence on processes which would be crucial in the control of anxiety-related behavior. However, (+)-WAY 100135 has been shown to induce a clear increase in noradrenaline release in the hippocampus (34) which would interfere with putative 5-HT-related anxiolytic-like effects in the present procedure. Finally, (+)-WAY 100135 at the largest dose (4 mg/kg), which did not alter overall responding when given alone, antagonized the effect of a small dose (0.125 mg/kg) of buspirone, but not that of a larger dose (0.25 mg/kg), in the present paradigm. This cannot be accounted for by differences in the affinity for 5-HT_{1A} receptors, since buspirone and (+)-WAY 100135 exhibit an equivalent nanomolar affinity for these binding sites on hippocampal membranes (13,27,51). It must be mentioned also that only a rather high dose of (+)-WAY 100135 (16 mg/kg SC) counteracted the reduction by 8-OH-DPAT (1 mg/kg SC) of the time spent immobile by mice subjected to the forced swimming test (M.H.T., unpublished results). These results contrast with the reported ability of (+)-WAY 100135 to easily antagonize a variety of behavioral and physiological indices of 5-HT_{1A} receptor activation such as 8-OH-DPAT-induced behavioral syndrome in rats and hypothermia in mice (13). Therefore, one can question the exact role of 5-HT_{1A} receptors [at least those which are easily blocked by (+)-WAY 100135 in vivo] in the release of responding during the Saf.CS−/Pun.CS− period.

Taken together, all these data do not strongly support the hypothesis of 5-HT_{1A} receptors as crucial targets for buspirone-like compounds to release the behavioral blockade in the safety signal withdrawal procedure, at least when these drugs are given acutely. The substratum which would subserve their action on suppressed behavior still remains to be precisely delineated.

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